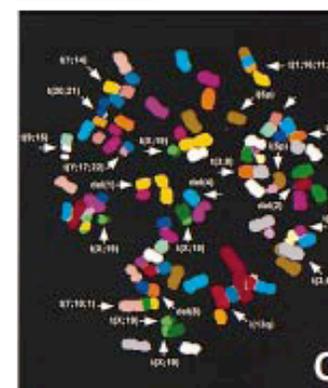
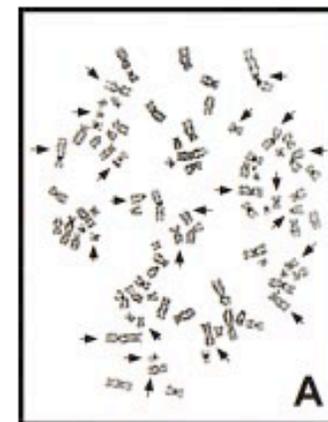
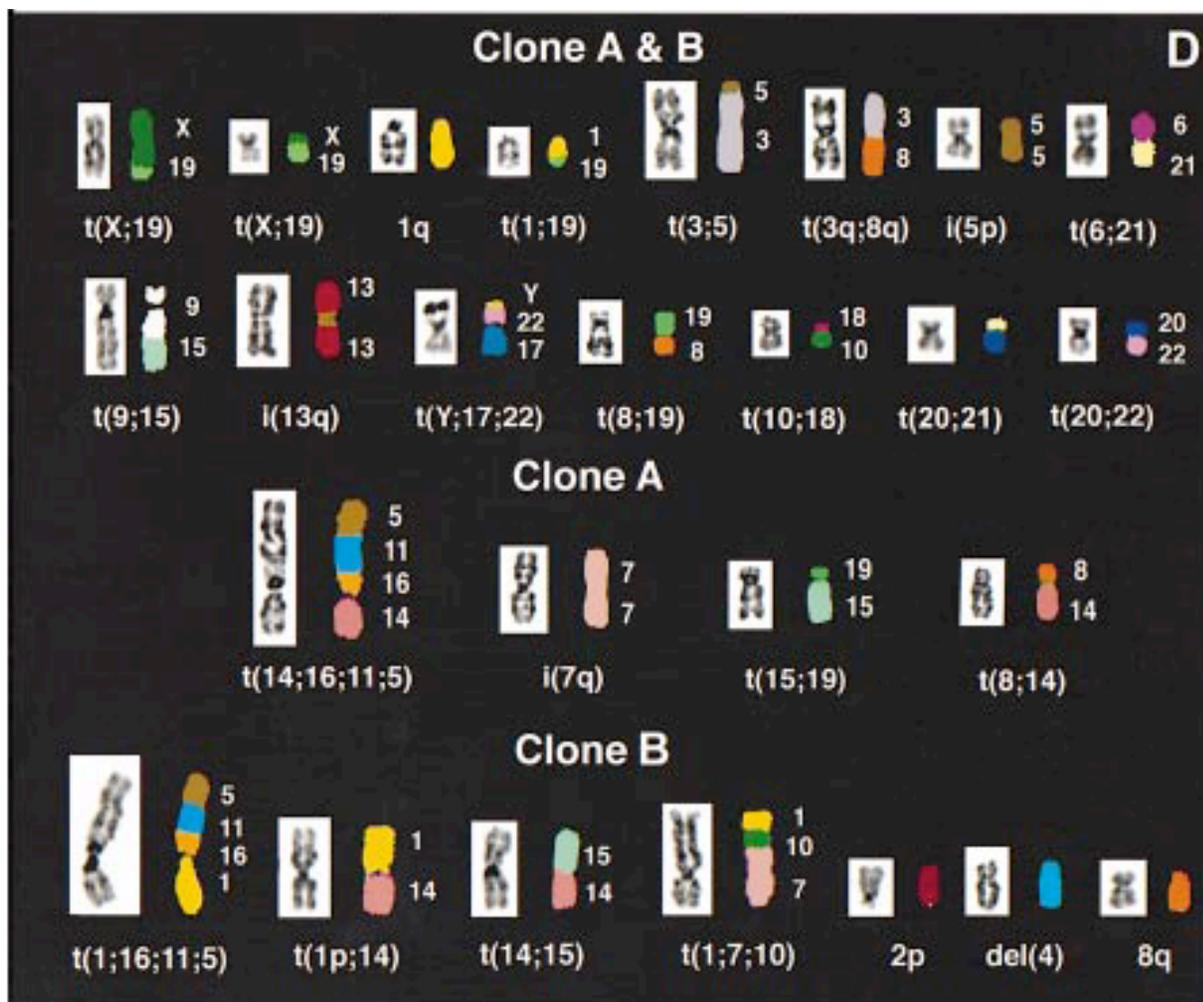


Suppression and Formation of
Gross Chromosomal Rearrangements
(Cross talks Between Different DNA Metabolism)

Kyungjae (KJ) Myung
Genome Instability Section
GMBB/NHGRI/NIH

Gross Chromosomal Rearrangements (GCRs) Visualized by SKY Analysis

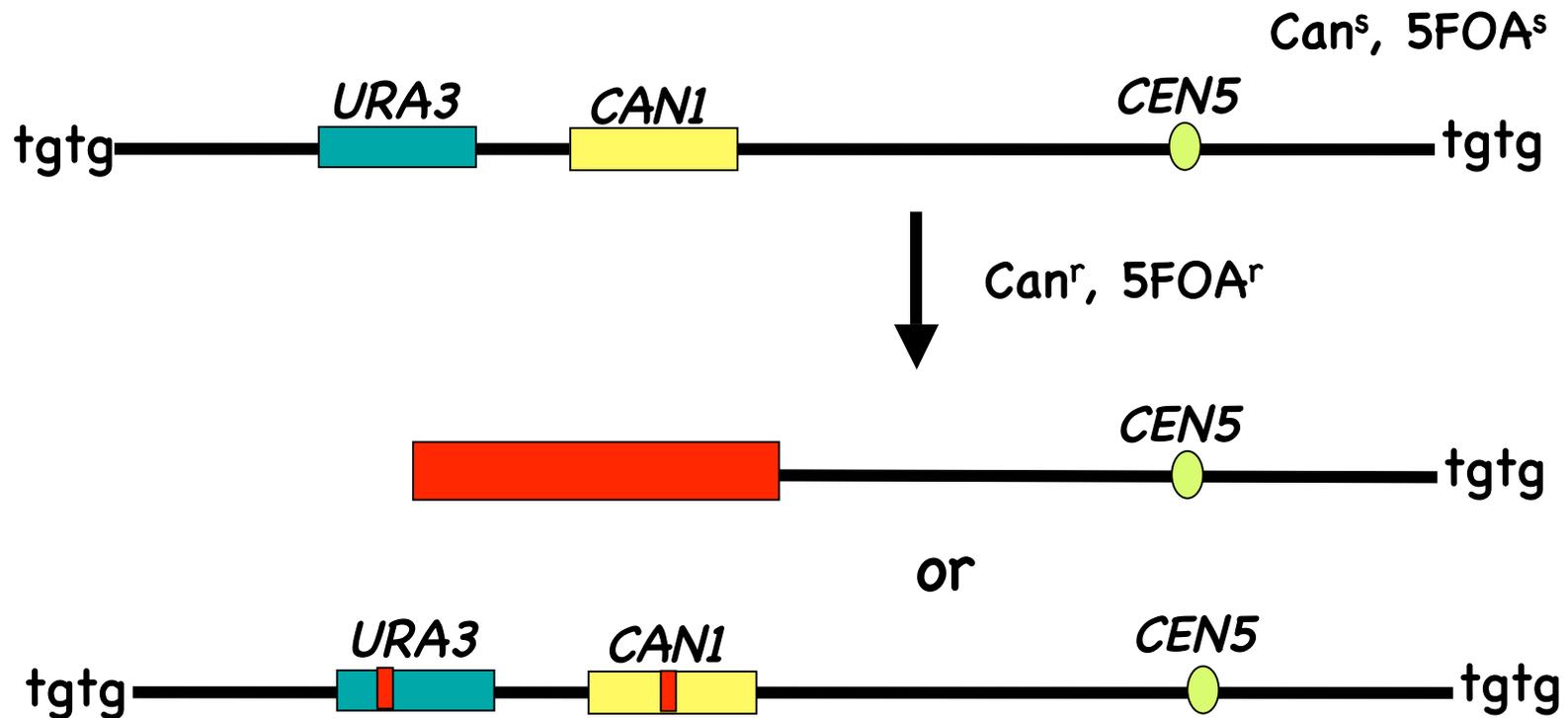


Padilla-Nash, H.M. et al.; *Genes, Chromosomes & Cancer*. **25**, 53 (1999)

1. Gross Chromosomal Rearrangements Assay
2. Rad1 and Rad10 in the Formation of GCR
3. Genome-wide Screening of GCR Mutator Genes
4. Recombination and Post Replication Repair function for the GCR formation.
5. Ku70/86's Role in the Suppression of GCR

GCR Assay

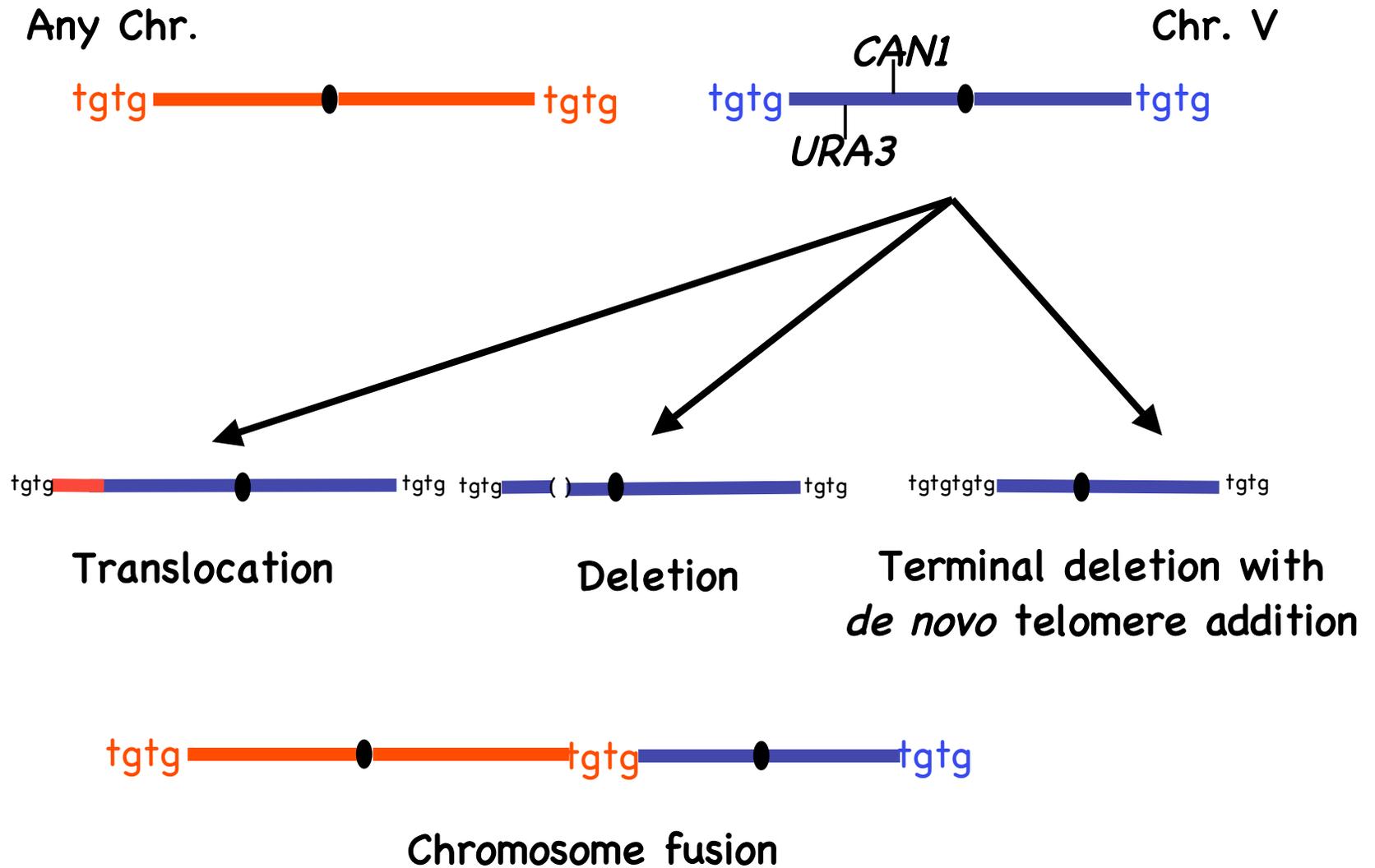
Assay for Measuring the Rate of Gross Chromosomal Rearrangements (GCRs)



Wild type rate of independent mutation = 10^{-12} to 10^{-14} per generation.

Wild type rate of genome rearrangements = 3.5×10^{-10} per generation.

GCRs Observed in Mutator Mutants



Rearrangement Breakpoints Structures

Terminal Deletion
& Telomere Addition

AGTAAATAAGACAGA *tgggtgtgggtgtgg*

Chr . V

Non-homology
Translocation

AAACAGCAAAGGCCA : CAGAACCGTATTCAT

CATTGTCATTATATT : CCATTTTCGCGTCTC

Chr . X

Micro-homology
Translocation

Chr . V

ATATTGGTATGATTG : CCCTTGGTGGTACTA

ATACTTGGCCGATTG : AACTTTTCATTGGT

Chr . I

Homeology
Translocation

Chr . V

CCAGGAGCCTGGGG : TCCAGGTATAATATC

TCCAGGAGCCTGGGG : GCCTGGCATTATCTC

Chr . XIV

Breakpoint Sequences in Cancer

RB Deletion

RB

TATAGCTTTTA . . (>135kb) . . CATGAATTTAACATAAA

BCR-ABL Translocation

BCR

GATTAGCCAGGCTAGGCAGT : GGGCACCTGTAATCACAACCTG
CCAAAGTTTGTCTACCCAGT : TTTAAATCCTGGCTTTCCCCT

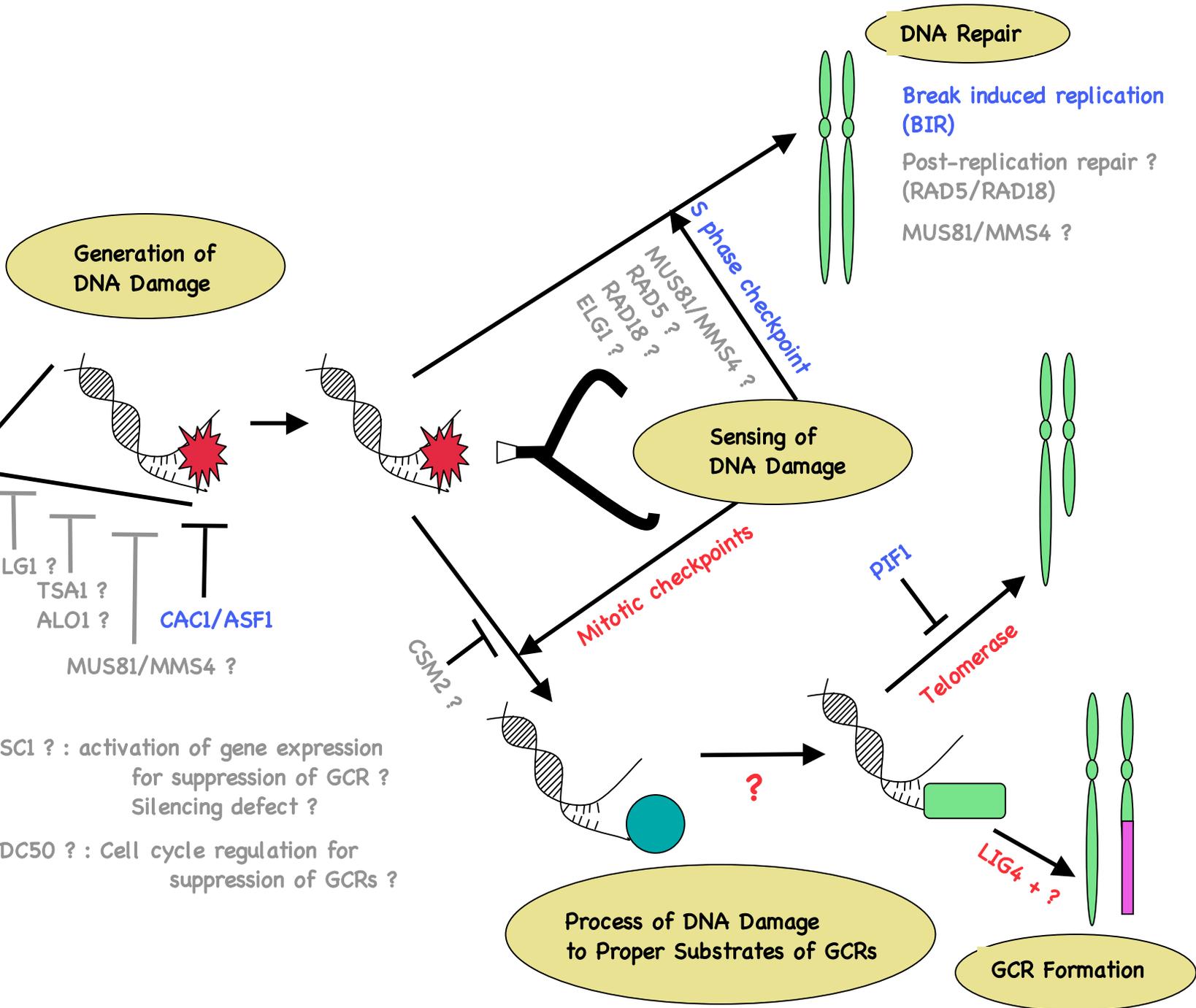
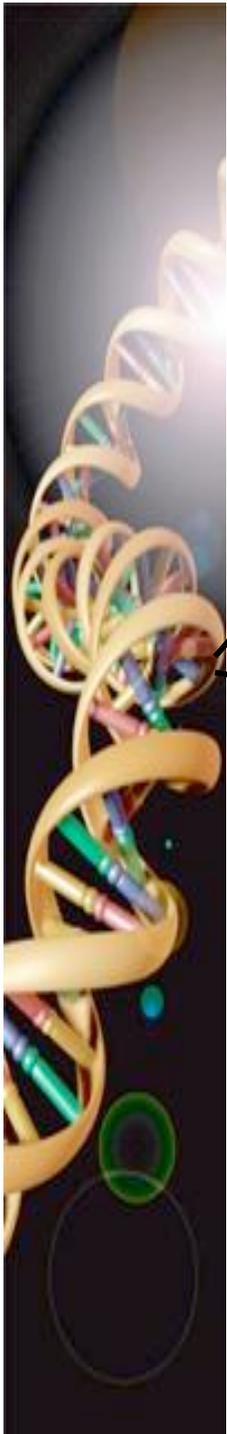
ABL

BCR

CTCATCGGGCAGGGTGTTGGG : GAAACAGGGAGGTTGTTTCAGA
AGTAAATTAAGGGTTATGGG : TCTTCACTTTCGTAGCTTCTA

ABL

From: J. G. Zhang et al., 1995; S. Canning & T. Dryja, 1989



Rad1 and Rad10

Radiation-sensitive mutants of *Saccharomyces cerevisiae*

Single-stranded DNA specific 5'-endonuclease

Heterodimer complex composed with Rad1 (~126 kDa, basic) and Rad10 (~24 kDa, acidic).

The Rad1 and Rad10 are required for damage-specific recognition and incision of DNA during **nucleotide excision repair**. Bardwell L et al., *Mol Cell Biol.* 12, 3041 (1992)

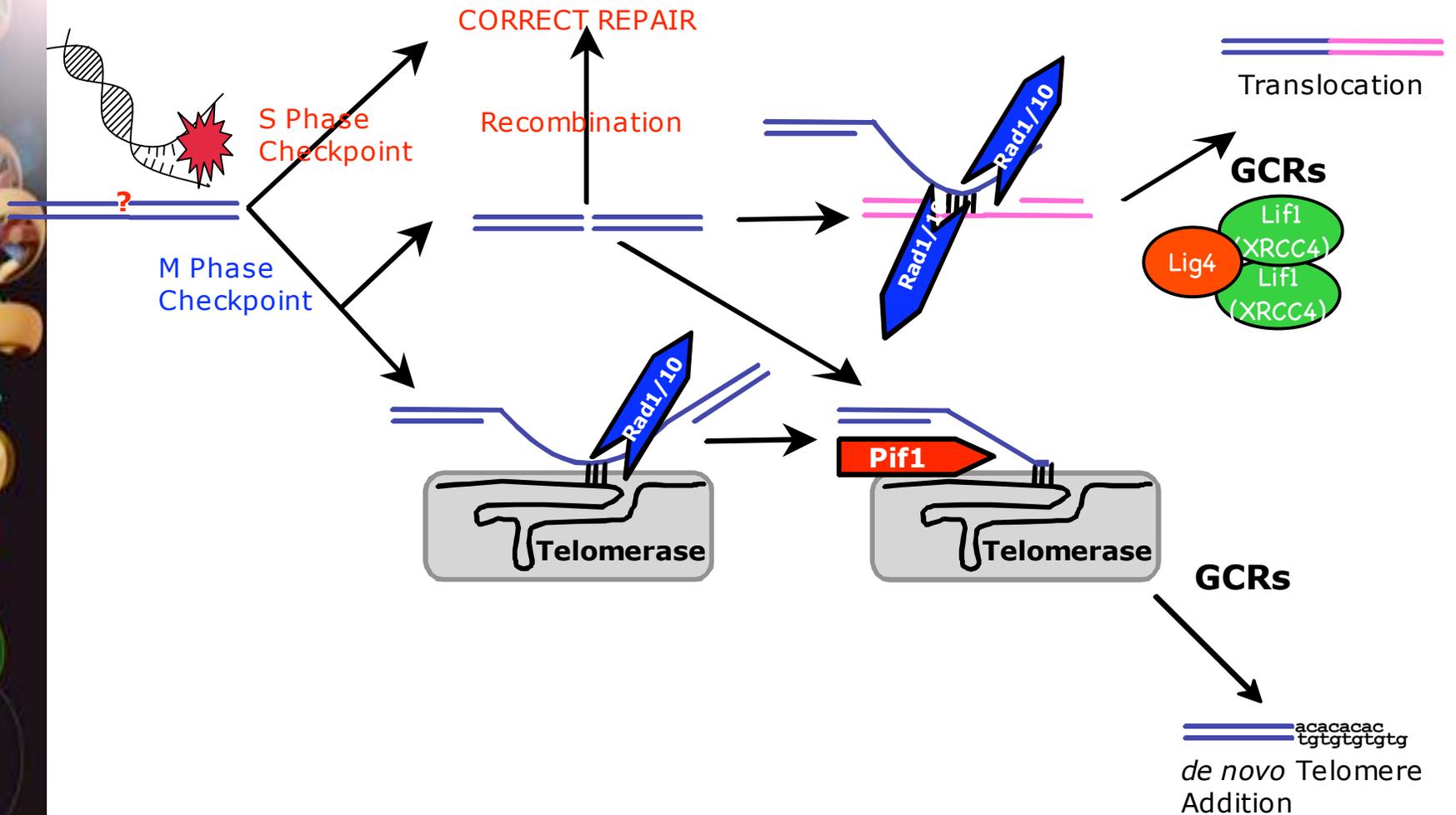
Rad1 and Rad10 also function in **recombination**. Schiestl RH and Prakash S., *Mol Cell Biol.* 8, 3619 (1988) & 10, 2485 (1990)

Human homologs are XPF (Rad1) and ERCC1 (Rad10) and XPF mutations have been documented in Xeroderma pigmentosum.

rad1 and *rad10* Mutations Reduced the GCR Rate in GCR Mutator Strains

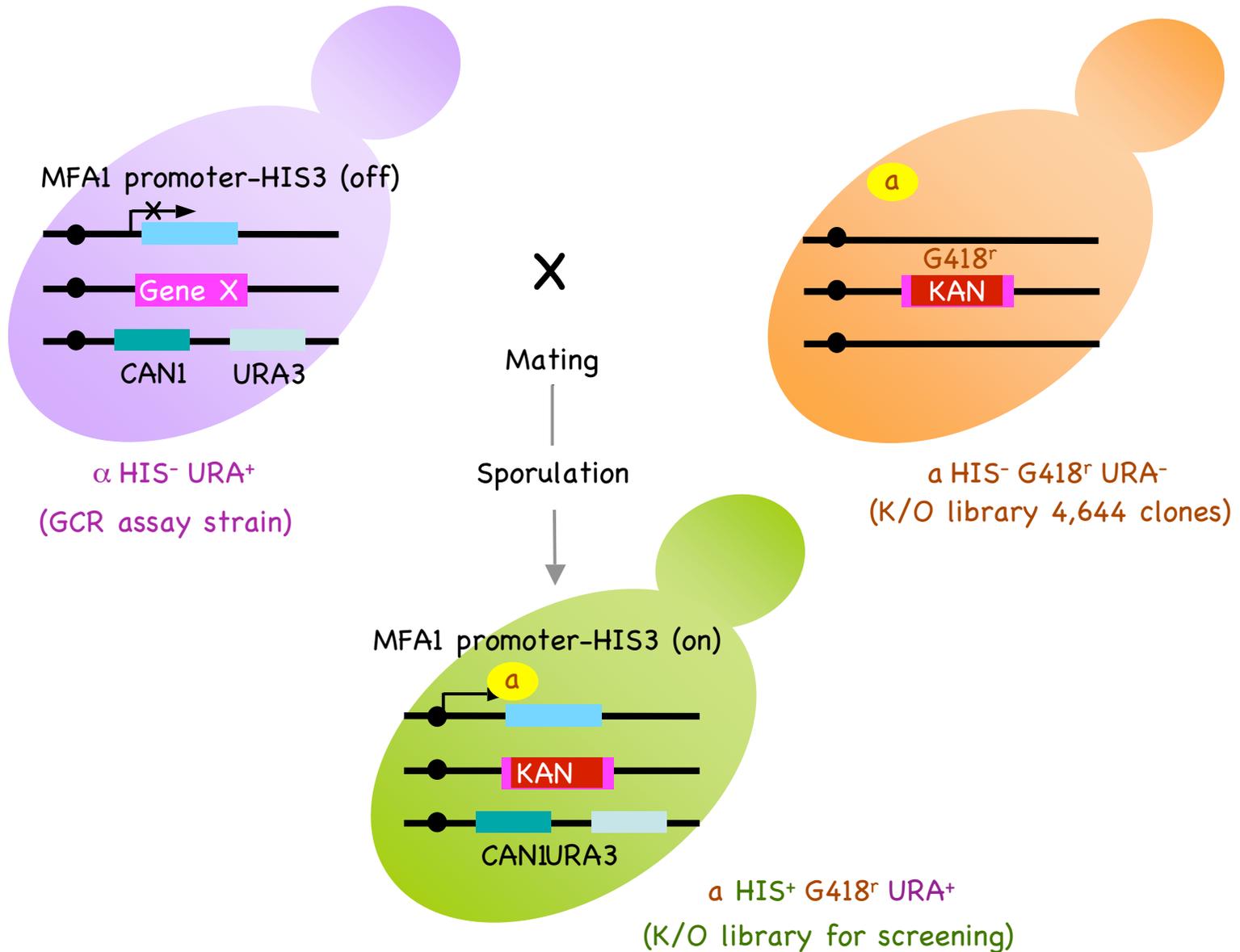
Genotype	Wild type	<i>rad1</i>	<i>rad10</i>
Wild type	3.5 × 10 ⁻¹⁰ (1)	3.8 × 10 ⁻¹⁰ (1)	3.5 × 10 ⁻¹⁰ (1)
<i>rfa1-t33</i> (replication)	4.7 × 10 ⁻⁷ (1343)	7.4 × 10 ⁻⁹ (21)	5.1 × 10 ⁻⁹ (15)
<i>mre11</i> (Multifunction)	2.2 × 10 ⁻⁷ (629)	3.9 × 10 ⁻⁸ (111)	6.0 × 10 ⁻⁸ (171)
<i>mec1</i> (Checkpoint)	6.4 × 10 ⁻⁸ (183)	5.4 × 10 ⁻⁹ (15)	2.0 × 10 ⁻⁸ (56)
<i>rad52</i> (Recombination)	4.4 × 10 ⁻⁸ (126)	2.2 × 10 ⁻⁹ (6)	2.8 × 10 ⁻⁹ (8)

Rad1 and Rad10 Function to Produce Better Structure for Spontaneous GCR Formation

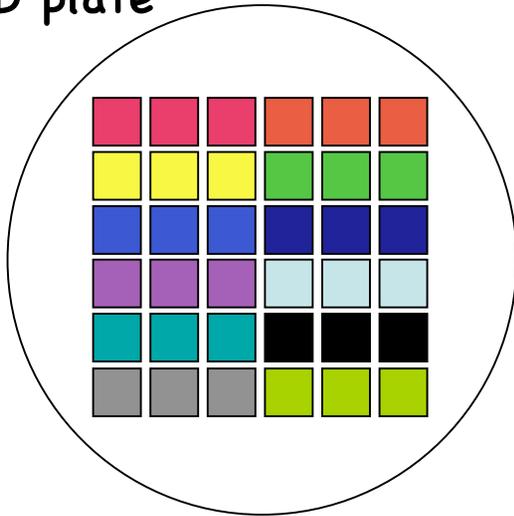


Genome-Wide Screening Of GCR Mutator Genes

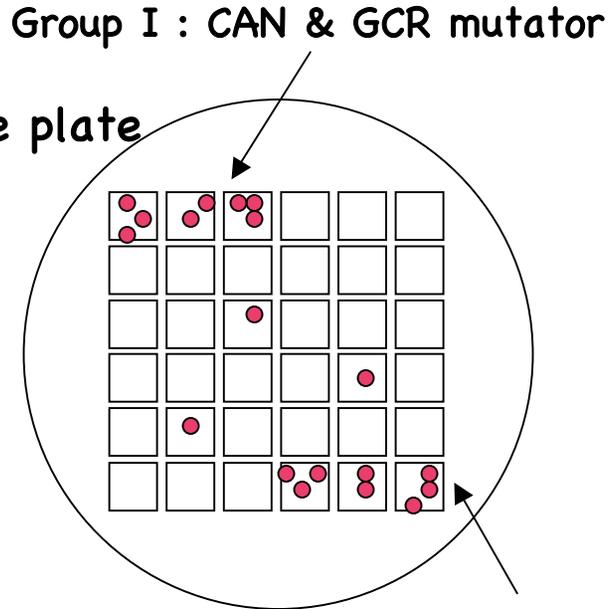
A Scheme for Screening of All Non-Essential Yeast Open Reading Frames for GCRs



YPD plate



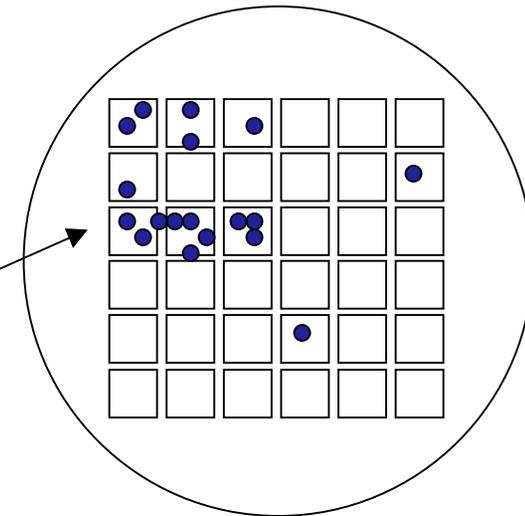
Canavanine plate



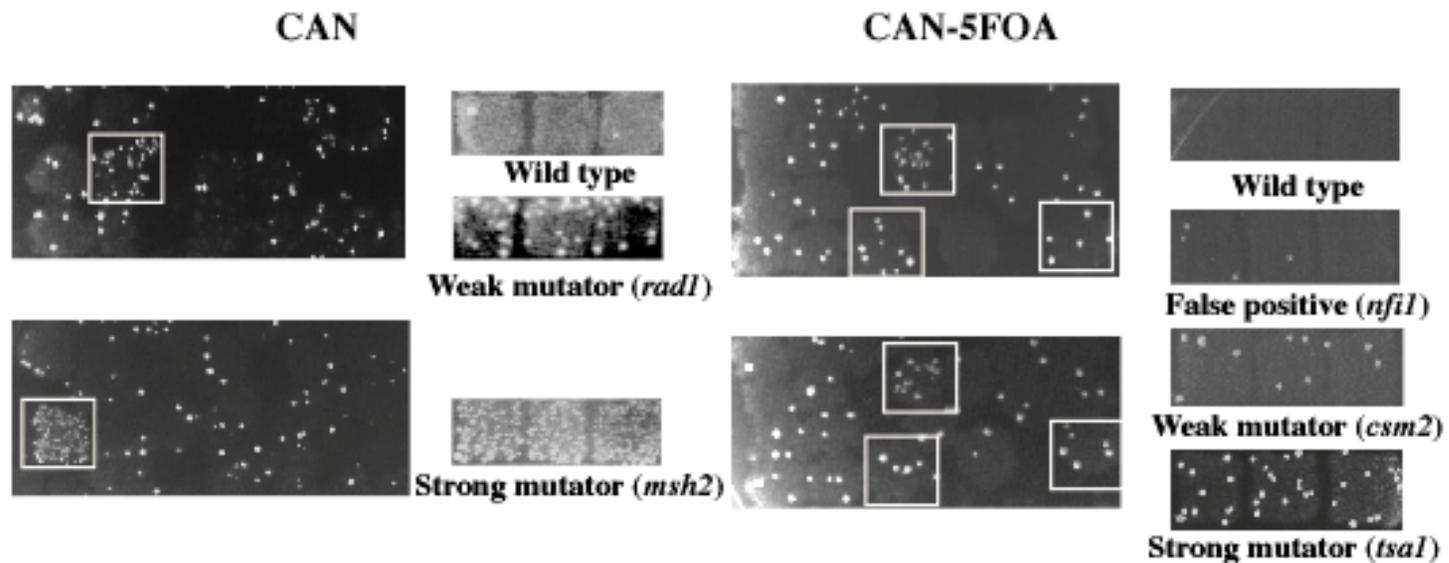
Canavanine & 5FOA plate

Group II :
CAN mutator

Group III : GCR mutator

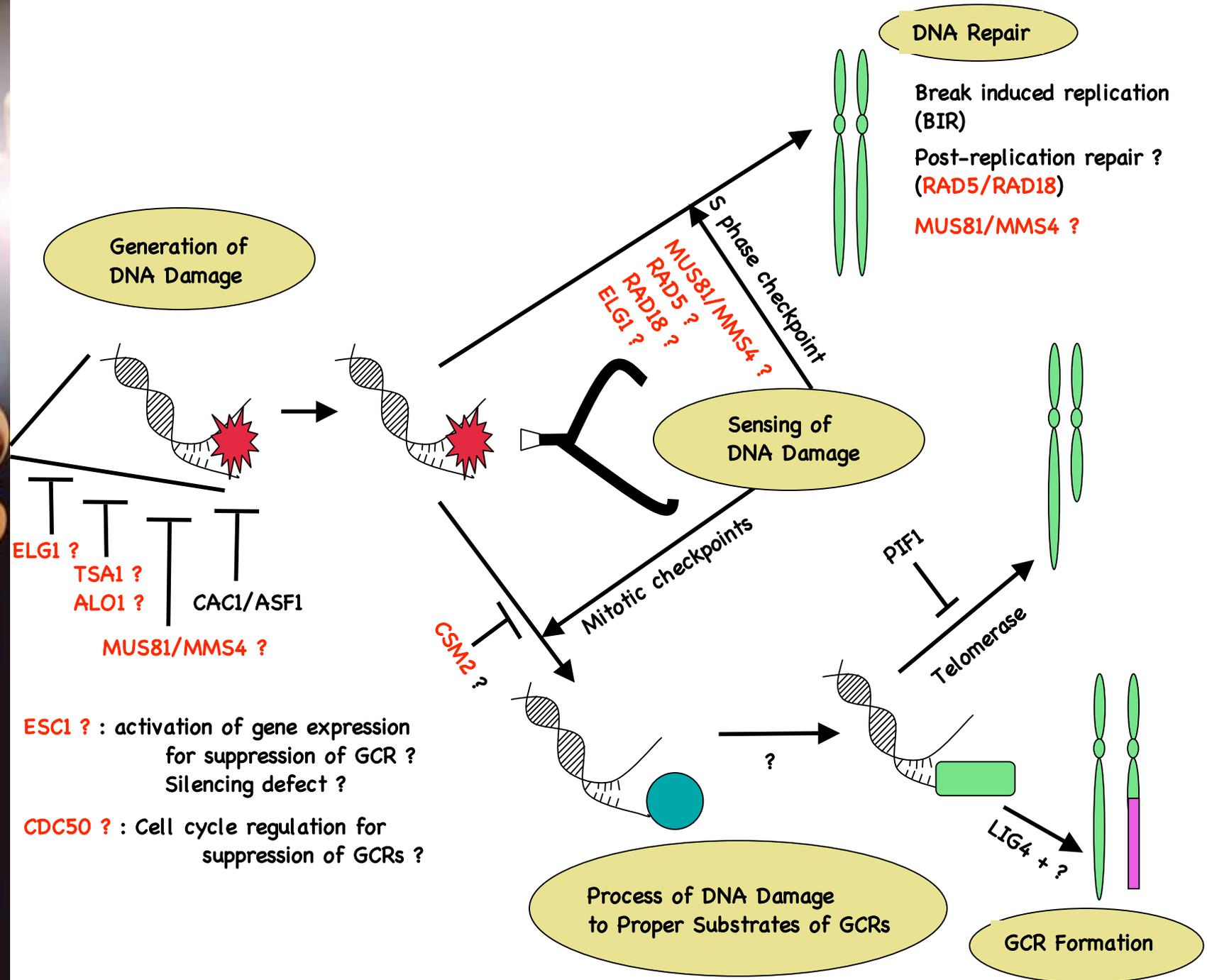


Mutators Increased the Number of Resistant Colonies

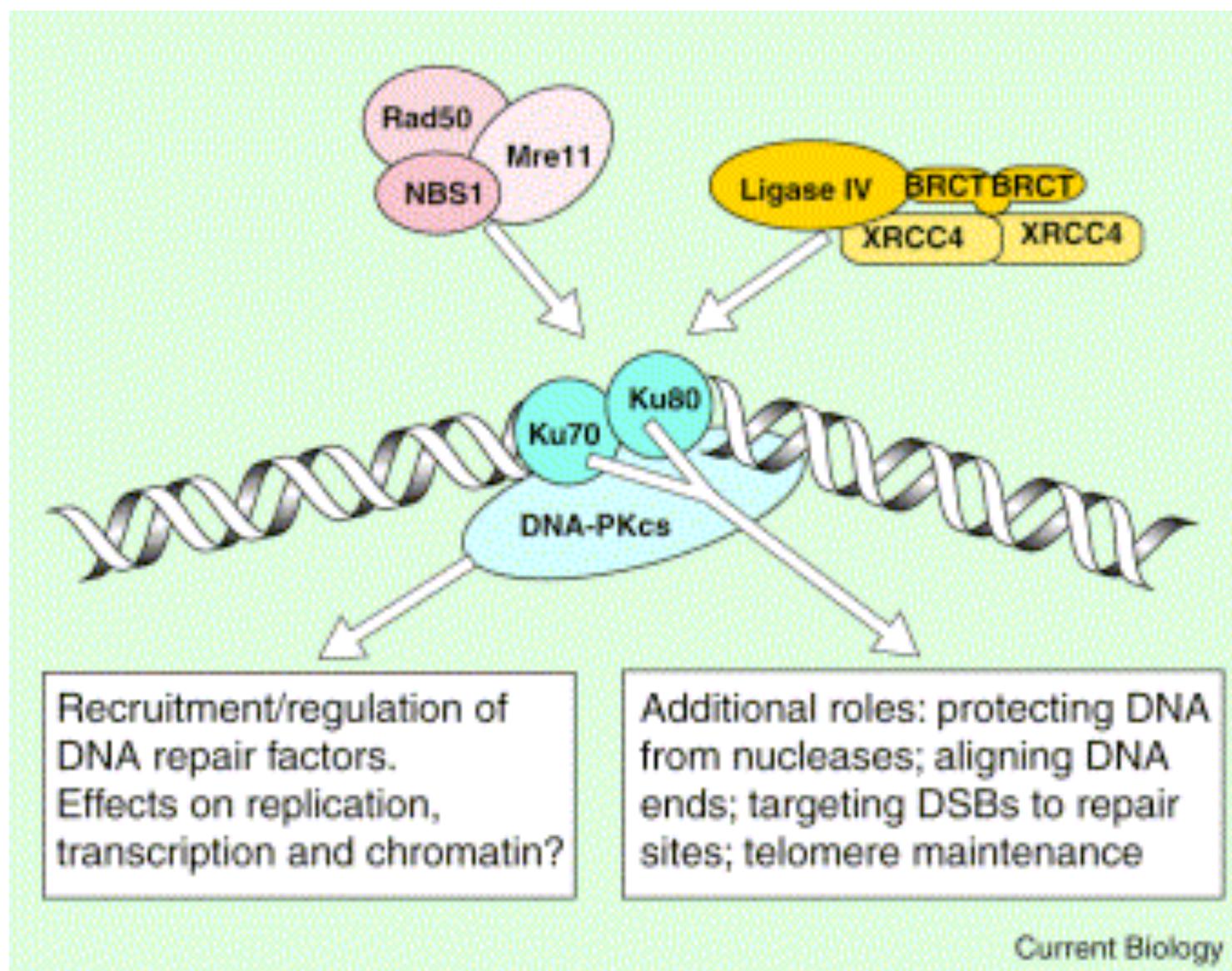


Effect of New GCR Mutator Gene Defects on the GCR Rates

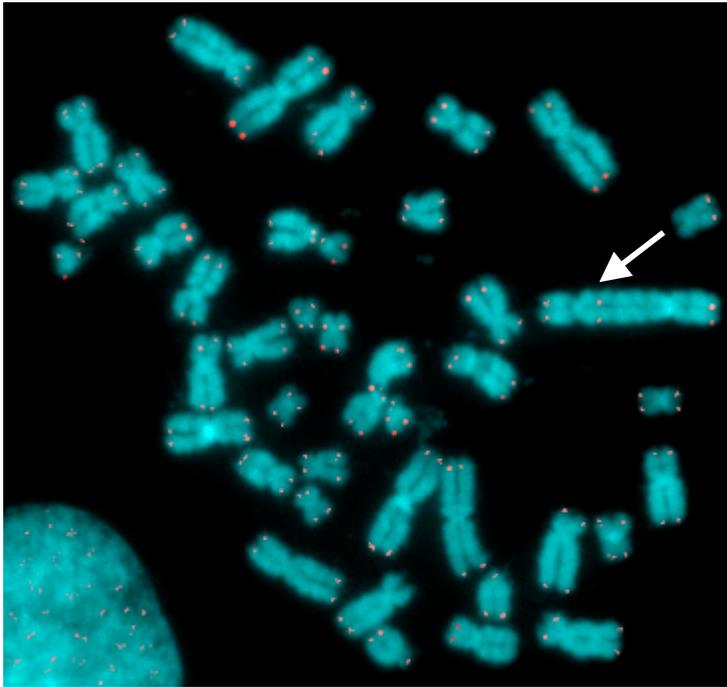
Relevant Genotype	WT	<i>pif1-m2</i>	
	GCR rate (CAN ^r -5FOA ^r)	GCR rate (CAN ^r -5FOA ^r)	
Wild type	3.5 x 10 ⁻¹⁰ (1)	6.3 x 10 ⁻⁸ (180)	
<i>alo1Δ</i>	4.7 x 10 ⁻⁸ (134)	1.1 x 10 ⁻⁷ (314)	
<i>cdc50Δ</i>	4.8 x 10 ⁻⁹ (14)	2.6 x 10 ⁻⁷ (743)	
<i>csm2Δ</i>	2.7 x 10 ⁻⁹ (8)	1.6 x 10 ⁻⁷ (457)	
<i>elg1Δ</i>	1.7 x 10 ⁻⁸ (49)	3.0 x 10 ⁻⁷ (857)	— de novo telomere addition (70%) + translocation (30%)
<i>esc1Δ</i>	2.3 x 10 ⁻⁹ (7)	1.1 x 10 ⁻⁷ (314)	
<i>mms4Δ</i>	5.9 x 10 ⁻⁸ (169)	2.3 x 10 ⁻⁷ (657)	
<i>rad5Δ</i>	6.3 x 10 ⁻⁸ (181)	2.2 x 10 ⁻⁷ (633)	— de novo telomere addition (100%)
<i>rad18Δ</i>	7.1 x 10 ⁻⁸ (202)	2.5 x 10 ⁻⁷ (714)	— de novo telomere addition (100%)
<i>tsa1Δ</i>	2.6 x 10 ⁻⁹ (7)	3.6 x 10 ⁻⁷ (1029)	
<i>ufo1Δ</i>	2.6 x 10 ⁻⁸ (74)	1.4 x 10 ⁻⁷ (400)	



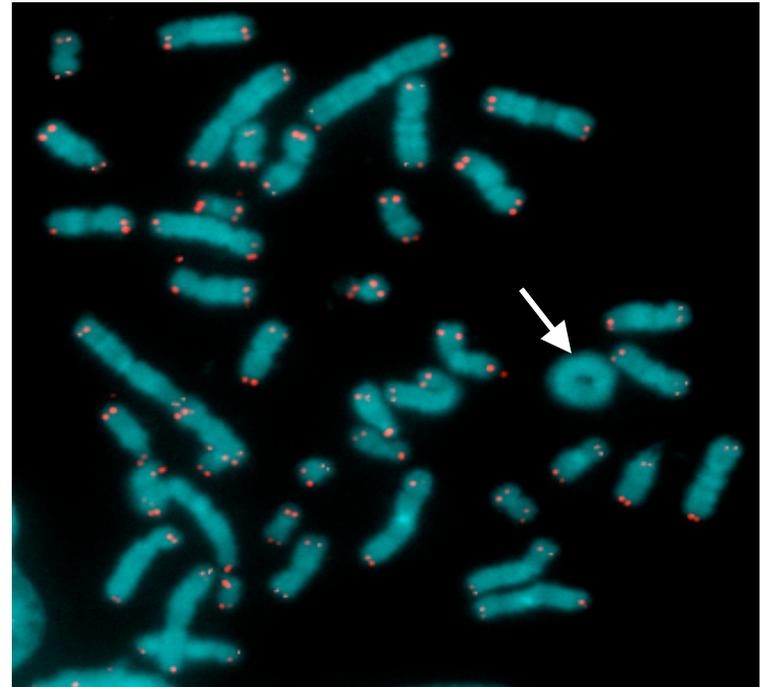
The GCR Suppression by Ku70 and Ku86



Gross Chromosomal Rearrangements were observed in Human Ku86^{+/-} Cell Line

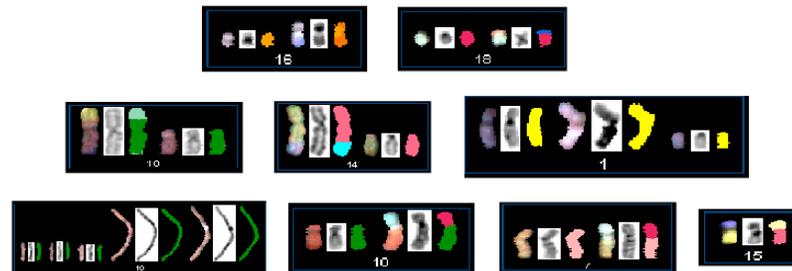
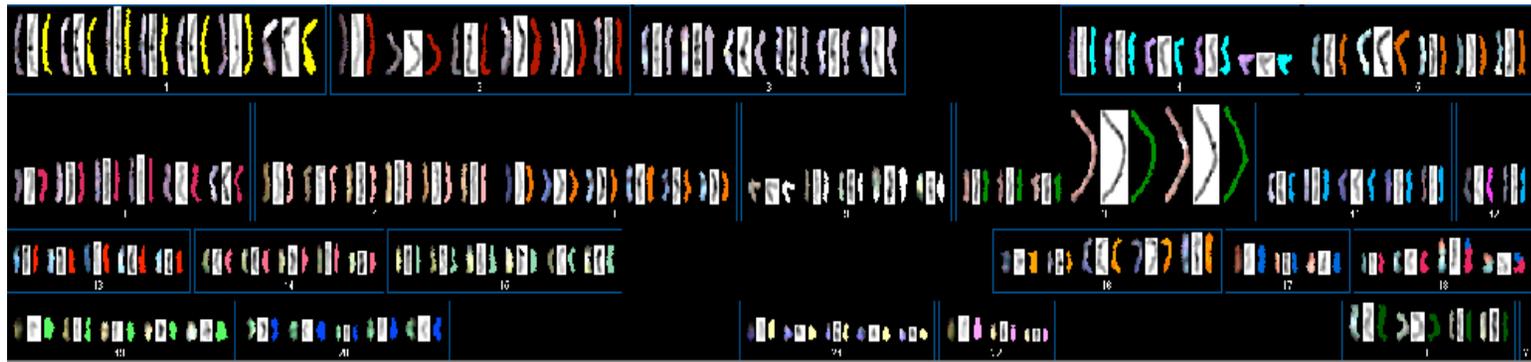
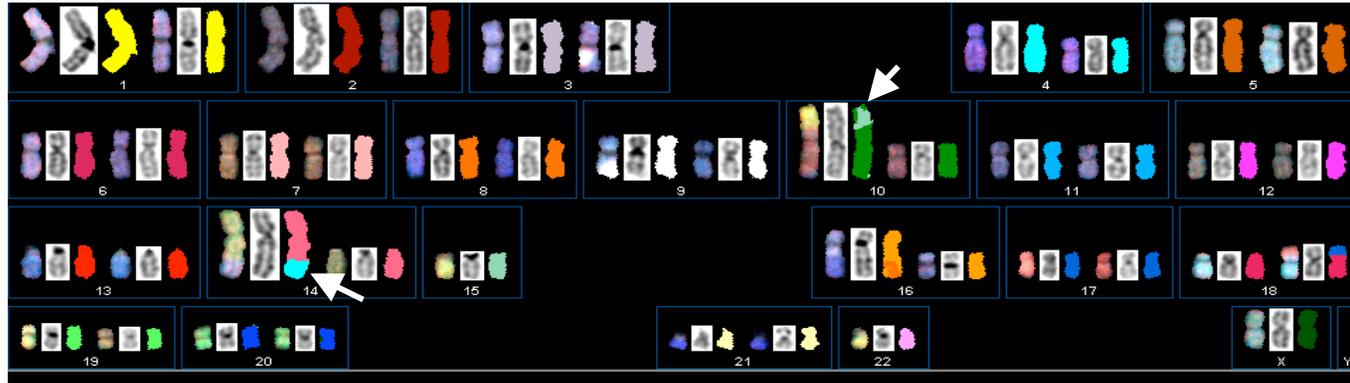


Chromosome Fusion



Ring Chromosome

GCRs observed in Human Ku86 +/- Cell Line



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